MOLECULAR GENETIC DIVERSITY OF MANIOC (*Manihot esculenta* CRANTZ) USING SIMPLE SEQUENCE REPEATED MARKERS (SSR) IN THE KARA REGION OF TOGO

ABSTRACT

Cassava (*Manihot esculenta* Crantz), a plant of the future, is one of the main crops with high genetic potential. Therefore, the knowledge of its genetic variability would be of great importance for its effective use in genetic improvement programs. The objective of this work was to characterize the genetic diversity of cassava cultivars present in the Kara region of Togo. Thus, a total of 91 cassava leaf samples collected in the 7 prefectures of the Kara region were analyzed using 7 pairs of SSR microsatellite primers. Fifty-three alleles were detected with an average of 7.57 alleles per locus. The polymorphic information content ranged from 0.63 to 0.83 with an average of 0.74, indicating a high level of marker polymorphism. Molecular analysis of variance revealed that the majority of variability occurred within individuals and accounted for 99 % of the total variation between individuals. Weak genetic differentiation (Fst=0.011) was also observed between populations. The genetic structuring model based on the *Neighbour-Joining* algorithm method divided the individuals into 6 groups independently of local names. These results indicate the presence of high genetic resource variability in the studied populations. Therefore, it would be important to implement management strategies to better conserve cassava genetic resources and to facilitate the identification of successful cultivars.

Keywords: cassava, genetic diversity, SSR marker, Togo

INTRODUCTION

Cassava (*Manihot esculenta* Crantz), the plant of the future is an important crop and food security resource for communities. It is one of the major sources of carbohydrates and the second most important source of starch in the world, just behind maize (Ceballos *et al*., 2015). With an estimated annual global production of 302.66 million tons (FAOSTAT, 2020); cassava feeds nearly 1 billion people (Reynolds, 2017). Africa alone produces more than 64% of the production (FAOSTAT, 2020) and is therefore the largest production area in the world. In Togo, cassava occupies an important place in the food production of local populations and is one of the most cultivated crops in all regions of the country in pure culture or in association with other crops. Moreover, according to the results of a survey in cassava production areas in Togo, a total of 168 cultivated cassava varieties were identified in only 40 villages (Kombate *et al*., 2017a).

Unfortunately, cassava plants are subject to abiotic and biotic constraints threatening the food security of millions of people (Tize *et al*., 2021); these plants are also subject to various evolutionary forces such as mutations, migration, hybridization, and polyploidization that are responsible for creating genetic variabilities (Colombo *et al*., 2000). Given the importance that this species presents; knowledge and exploitation of its diversity is paramount for the maintenance and improvement of its productivity (Adjebeng-Danquah *et al*., 2016).

Morphological markers were the first to be used to measure genetic variation as well as desired traits (Anjum *et al*., 2018) in cassava but have proven unsatisfactory, as they have only a small number of distinctive characters and do not allow for accurate classification (Porth & El-Kassaby, 2014; Singh *et al*., 2018).

In response to this situation and in order to increase the efficiency of identification and recombination of desired traits, a number of molecular resources and tools have been developed in recent years. Among these molecular tools, microsatellites or SSR (Simple Sequence Repeat) markers are considered as markers of choice, due to their many attributes such as multi-allelic nature, codominant inheritance, high reproducibility, relative integration, random distribution in the genome, (Agarwal *et al*., 2008; Kalia *et al*., 2011; Varshney *et al*., 2005) and ease of development of cost-effective protocols and assays (Singh *et al*., 2019).

The objective of this study was to characterize and access the extent of genetic diversity among 91 cassava cultivars collected in the Kara region using SSR markers.

Material and Methods

Study site and collection of leaf samples

A survey was conducted in cassava fields at least 10 km apart across the seven prefectures (Dankpen, Keran, Bassar, Kozah, Assoli, Bimah, Doufelgou) of the Kara region of Togo, a West African country bordering the Gulf of Guinea (Figure 1). The Kara region lies between parallels 9 °25 and 10 °10 north latitude and meridians 0 °15 and 1 °30 east longitude. A total of 91 cassava (*Manihot esculenta* Crantz) leaf samples were collected and divided into 5 cultivars according to the names provided by the farmers (Table 2). These samples were placed in paper envelopes, labeled and transported from the field to the Laboratory of Virology and Plant Biotechnology. For molecular analysis, the samples were oven dried at 40 °C for 24 hours.

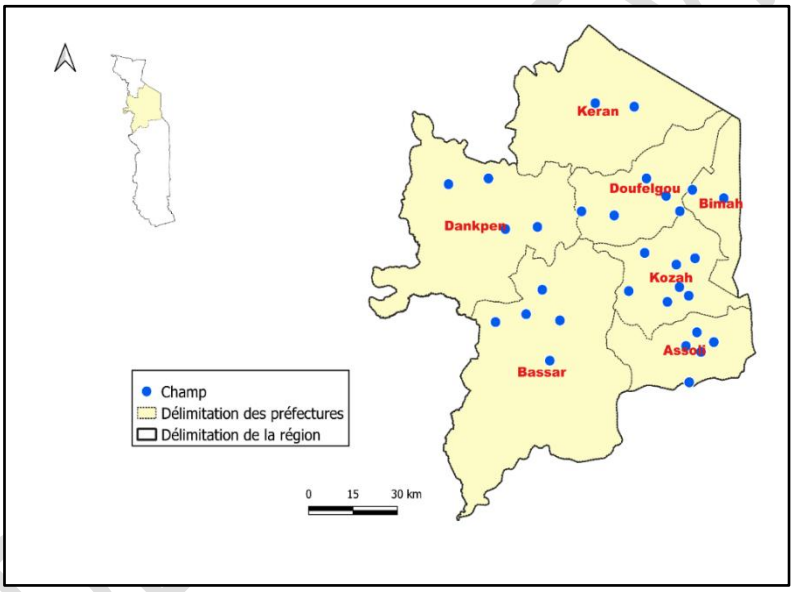

Figure 1: Map of the sampling area

Table 1: Cultivars collected in Kara region

Local name	Number
Akpadjin	17
Fétonégbodji	11
Ganave	34
Gbazekouté	17
Yovovidjin	12
Total	**91**

Extraction of genomic DNA

DNA was extracted from 100 mg of dried and ground leaves per sample from the InnuPREP Plant DNA kit following the manufacturer's recommendations at the Laboratory of Plant Virology and Biotechnology (LVBV). In summary:

- ✓ 400 µl SLS lysis solution and 20 µl proteinase K are added to the ground sheet in a 1.5 ml tube; the whole is vortexed vigorously for 5 seconds and incubated at 65 °C for 30 minutes;
- ✓ the shredded material is transferred to a pre-filter and centrifuged at 11,000 rpm for 1 minute; 200 µl of SBS binding solution is added after centrifugation to the lysed sample and mixed and centrifuged;
- ✓ 650 µl of MS wash solution, to the spin filter and centrifuged at 11,000 rpm for 1 minute. The washisrepeatedtwice;
- ✓ the spin filter is placed in an elution tube to which 200 µl of elution buffer is added and incubated at room temperature for 1 minute then centrifuged at 11,000 rpm for 1 minute. The extracted DNA was stored at 20 °C for molecular analysis.

DNA amplification by polymerase chain reaction (PCR)

A total of 7 microsatellite (SSR) primer pairs, widely distributed in the cassava genome (Mba *et al*., 2001), were used (Table 2). PCR amplification was performed in a thermal cycler (Applied biosystems) using the method of Mba et *al*. (2001) described as follows: an initial 1 min denaturation at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 seconds, hybridization at 55 °C for 1 min, elongation at 72 °C for 1 min, and finally a final elongation for 10 min at 72 °C.

Locus	Amorce Forward (5' ⟶ 3')	Amorce reverse (5'3')	Taille (pb)

Table 2 : Primer pairs for microsatellite markers (Mba*et al*., 2001)

SSRY9	ACAATTCATCATGAGTCATCAACT	CCGTTATTGTTCCTGGTCCT	278
SSRY12	AACTGTCAAACCATTCTACTTGC	GCCAGCAAGGTTTGCTACAT	266
SSRY21	CCTGCCACAATATTGAAATGG	CAACAATTGGACTAAGCAGCA	192
SSRY69	CGATCTCAGTCGATACCCAAG	CACTCCGTTGCAGGCATTA	239
SSRY100	ATCCTTGCCTGACATTTTGC	TTCGCAGAGTCCAATTGTTG	210
SSRY161	AAGGAACACCTCTCCTAGAATCA	CCAGCTGTATGTTGAGTGAGC	220
SSRY181	GGTAGATCTGGATCGAGGAGG	CAATCGAAACCGACGATACA	199

Gel Electrophoresis

PCR products were analyzed by 1.5 % agarose gel electrophoresis stained with ethidium bromide (0.5 µg/mg) in 1 X TBE buffer for 90 minutes using 10 µl of PCR products. The gel was visualized under UV light.

Data Analysis.

Genetic diversity was analyzed using various programs. Intra-population genetic diversity parameters, namely number of alleles per locus (Na), allelic frequencies (fa), observed heterozygosity (Ho), genetic diversity or expected heterozygosity (He) (Nei, 1978), and polymorphic information content (PIC) value were estimated with Power Marker Version 3.25 software (Liu & Muse, 2005). Polymorphism rate (% P) and fixation index (Fis) were calculated using GenAlEx v6.502 software (Smouse &Peakall, 2012). For inter-population diversity, the Differentiation Index (Fst) and Molecular Analysis of Variance (AMOVA) were the two approaches used. These approaches were performed on the raw data matrix using 999 permutations using GenAlEx v6.502 software (Smouse &Peakall, 2012).

Finally, a phylogenetic tree was constructed using the *Neighbour-Joining* algorithm method (Nei, 1973) based on the genetic similarity distance matrix to determine genetic structure. The robustness of the tree nodes was estimated with a bootstrap analysis using 1000 permutations using DARwin v6.0.21 software (Perrier *et al*., 2003).

Results and Discussion

Detection of loci within cultivars

The PCR results of the 91 individuals showed the presence of the desired loci within the cultivars studied. Individuals 3, 13, 21 all belonging to the cultivar Ganave did not react positively to the 7 pairs of marker primers, so these samples were not considered; therefore, the study was based on the results of the remaining 88 individuals. Figure 2 shows an example of an electrophoretogram obtained with the SSRY69 primer pair.

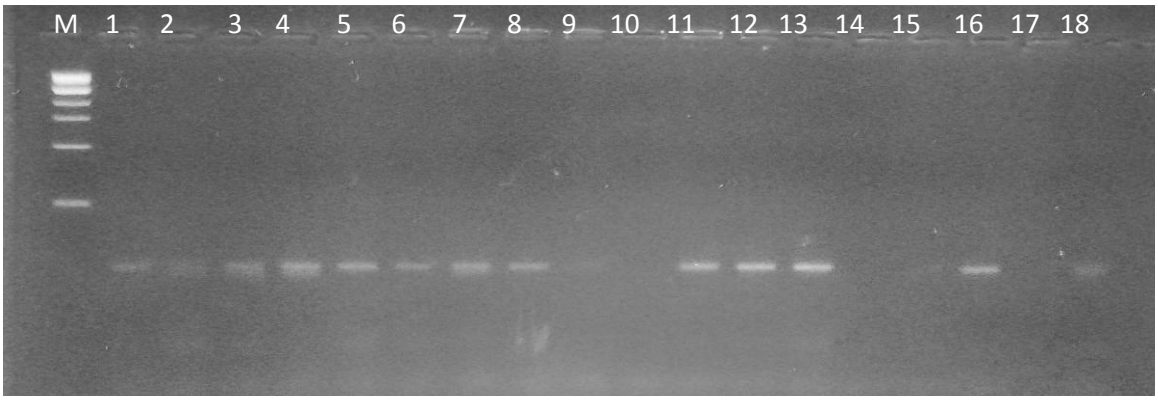

Figure 2: Electrophoretogram showing the results obtained with the SSRY69 primer pair on 18 samples. M = marker; sequence number of positive samples: 1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 13, 16, 18; sequence number of negative samples: 9, 10, 14, 15, 17.

Analysis of SSR marker polymorphisms

In this study, the 7 SSR marker primer pairs used amplified 1 or 2 fragments (alleles) per genotype. Conventionally, genotypes showing only 1 amplified allele are considered homozygous, whereas in the case of the presence of 2 alleles, the genotype is considered heterozygous. For all individuals in the study, a total of 53 alleles were observed ranging from 7 alleles (SSRY69, SSRY100, and SSRY181) to 8 alleles (SSRY9, SSRY12, SSRY21, and SSRY161) with an average of 7.57 alleles per locus (Table 3).

The average allele frequency was 0.35 and ranged from 0.25 for SSRY9 to 0.53 for SSRY69. Calculated expected heterozygosity He (genetic diversity) ranged from 0.67 for SSRY69 to 0.84 for SSRY9 with a mean of 0.77, while observed heterozygosity Ho ranged from 0.02 for SSRY12 and SSRY181 to 0.40 for SSRY100 with a mean of 0.17.

Table 3: Estimated diversity revealed by SSR markers

Locus	N	Na	fa	Ho	He	PIC
SSRY9	32	8	0,25	0,13	0,84	0,83
SSRY12	46	8	0,27	0,02	0,83	0,81
SSRY21	52	8	0,33	0,29	0,77	0,74
SSRY69	49	7	0,53	0,16	0,67	0,63
SSRY100	25	7	0,34	0,40	0,77	0,74
SSRY161	59	8	0,36	0,20	0,74	0,70
SSRY181	57	7	0,33	0,02	0,78	0,75
Mean	45,71	7,57	0,35	0,17	0,77	0,74

N= Number of individuals, Na = Number of alleles, Ho = Observed heterozygosity; He = Expected heterozygosity; PIC = Polymorphic information content, fa= Allelic frequency

The average observed heterozygosity was similar to the results obtained by Kouakou et *al*. (2022) (Ho = 0.166) who evaluated 288 accessions from several regions of Côte d'Ivoire with 11 SSR markers. These observed heterozygosity values are low compared to those reported in the studies of Adjebeng-Danquah et *al*. (2020), which is equal to 0.42. Kouakou et *al*. (2022) reported that a low Ho value could be due to the use of fewer accessions and SSR markers. This was found in this study.

A deficit in observed heterozygosity was also detected in studies conducted on cassava samples from the Brazilian Amazon and this deficit is thought to be a consequence of asexual propagation and immediate fixation of genotypes (Mühlen*et al*., 2013).

Further analysis of the polymorphic alleles of each SSR primer pair used demonstrated the level of informativeness of the primers that was determined by calculating the polymorphic information content (PIC). The PIC of the primer pairs ranged from 0.63 for SSRY69 to 0.83 for SSRY9, with an average of 0.74 (Table 3). Therefore, all 7 loci were highly informative (PIC > 0.5), indicating the effectiveness of the microsatellite markers used.

This is in agreement with the results of other studies in cassava, where the SSRs tested were highly polymorphic (Turyagyenda*et al*., 2012). Furthermore, among the 26 SSRs used in the

study by Turyagyendaet *al*. (2012); the pairs SSRY9, SSRY12, SSRY21, SSRY69, SSRY100, SSRY161, and SSRY181 exhibited PICs of 0.620, 0.597, 0.743, 0.775, 0.781, 0.723, 0.627 respectively.

Genetic diversity within populations

The number of individuals per population ranged from 11 (Fétonégbodji) to 31 (Ganave) (Table 4). More than one allele was detected in each population. The Ganave population was the richest with an average number of alleles of 5.14 per locus.

Table 4: Estimates of genetic diversity and fixation index by population

Population	N	Na	Ho	He	% P	Fis
Akpadjin	17	5	0,27	0,72	100	0,61
Fétonégbodji	11	3,43	0,25	0,62	100	0,53
Ganave	31	5,14	0,17	0,71	100	0,73
Gbazekouté	17	4,86	0,17	0,72	100	0,75
Yovovidjin	12	3,71	0,11	0,55	85,71	0,80
Mean	17,60	4,43	0,19	0,66	97,14	0,68

N= Number of individuals, Na = Number of alleles, Ho = Observed heterozygosity; He = Expected heterozygosity; Fis = Fixation index; % P = Polymorphism rate

Among the five populations analyzed, the polymorphism of the seven markers was 100 % for the cultivars Akpadjin, Fétonégbodji, Ganave and Gbazekouté. On the other hand, a low polymorphism rate of 85.71 % was observed for the cultivar Yovovidjin showing that a number of markers were monomorphic for this cultivar. Thus; the 5 cassava populations studied generated an average of 97.14 % polymorphism rate. These results are in agreement with the results of Carrasco (2012) and Mühlenet *al*. (2000) who obtained 95 % and 97.96 % polymorphic loci, respectively, when assessing the genetic diversity of cassava records based on microsatellite markers.

Expected heterozygosity values were higher for all populations compared to observed values, generating positive values for the fixation index that ranged from 0.53 (Fétonégbodji) to 0.80 (Yovovidjin). The inbreeding coefficient (fixation index) presented an average value of 0.68

indicating that the alleles of the populations studied are fixed by the inbreeding process. This result is confirmed by the inferiority of the observed mean heterozygosity value (0.19) compared to the expected mean heterozygosity value (0.66).

The overall fixation index results could be related to agricultural practices such as regeneration of cuttings. In addition, studies by (Kombate *et al*., 2017b) in Togo showed that the majority (48.1%) of cuttings used were from previous cassava crops. This could also explain the high inbreeding rate observed.

Inter-population diversity

The estimated percentage of variation between populations was determined by molecular analysis of variance (AMOVA) (Table 5).

Table 5: Molecular analysis of variance (AMOVA) within the 88 cassava cultivars using the 7 SSR markers

Source of variation	Df	SS	MS	Est. Var	%	F-Statistics(Fst)
Between populations	4	21,54	5,38	0,02	1 %	0,011
Betweenindividuals	83	373,28	4,49	2,10	87 %	
Withinindividuals	88	26	0,29	0,29	12 %	
Total	175	420,83		2,42	100 %	

Df: degree of freedom; SS: sum of squares, MS: mean of sums of squares; Var.Est: estimated variance; %: percentage of variation, Fst: differentiation index

The AMOVA results revealed that over 80 % of the total genetic diversity is between individuals in the region, while 1 % and 12 % of this diversity is attributed to differences between populations and within individuals, respectively.

Based on the threshold values reported by Wright (1978), low differentiation (Fst = 0.011) was obtained between populations in the present study. This Fst value implies that a large proportion (99%) of the total genetic variability is explained by within-population variation.

These results could be explained by high gene flow between populations due to the continuous exchange of planting material among farmers in the region. On the other hand, this may be related to the close proximity of the surveyed areas (10 km). Furthermore, studies by Nosil*et al*. (2008) have shown that the main drivers of genetic differentiation and thus genetic diversity between plant populations are geographical distances and the environment.

Genetic structure of cassava cultivars

The structuring model based on the *Neighbour-Joining* algorithm (Nei, 1973) was used to divide the 88 individuals into six groups (Figure 3).

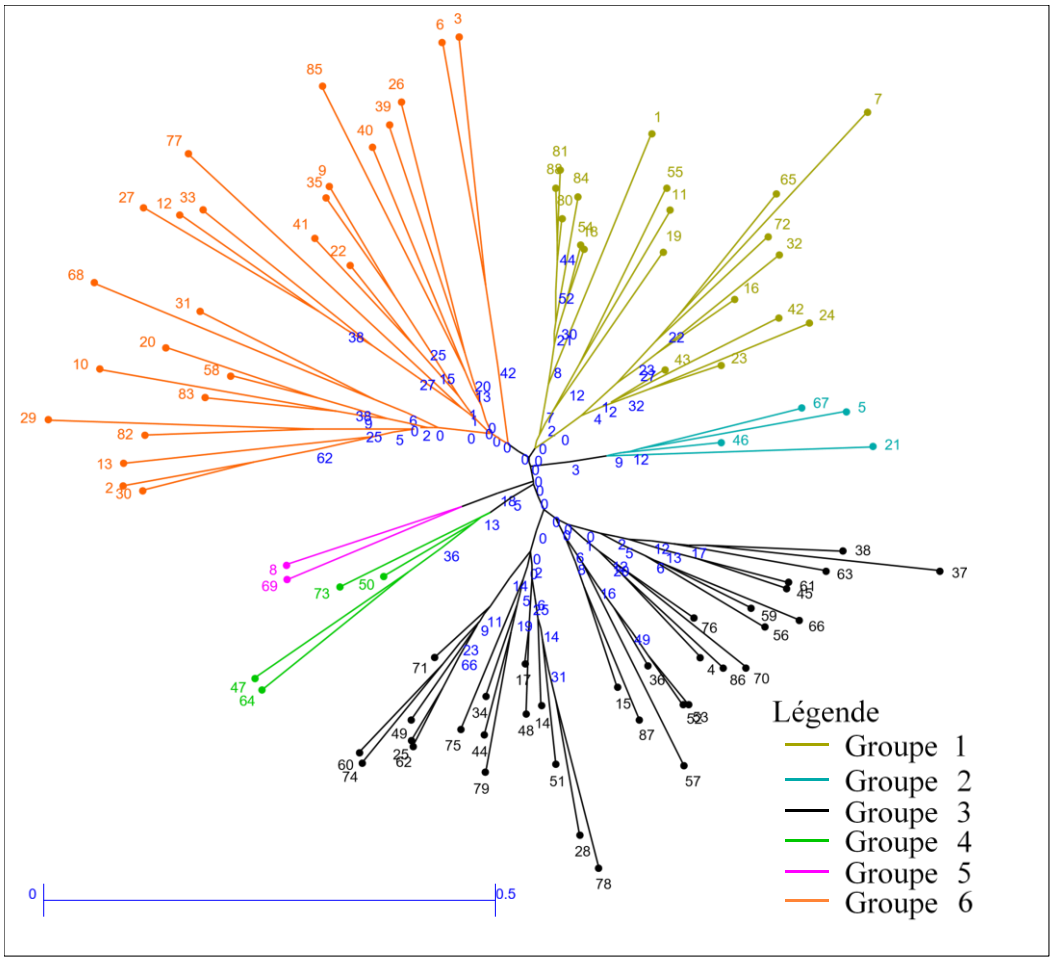

Figure 3 :Phylogenetictreeshowinggeneticrelationshipsamong 88 cassava cultivars based on genetic distance

Figure 4 derived from the dendrogram analysis shows the distribution of cultivars within the genetic groups created.

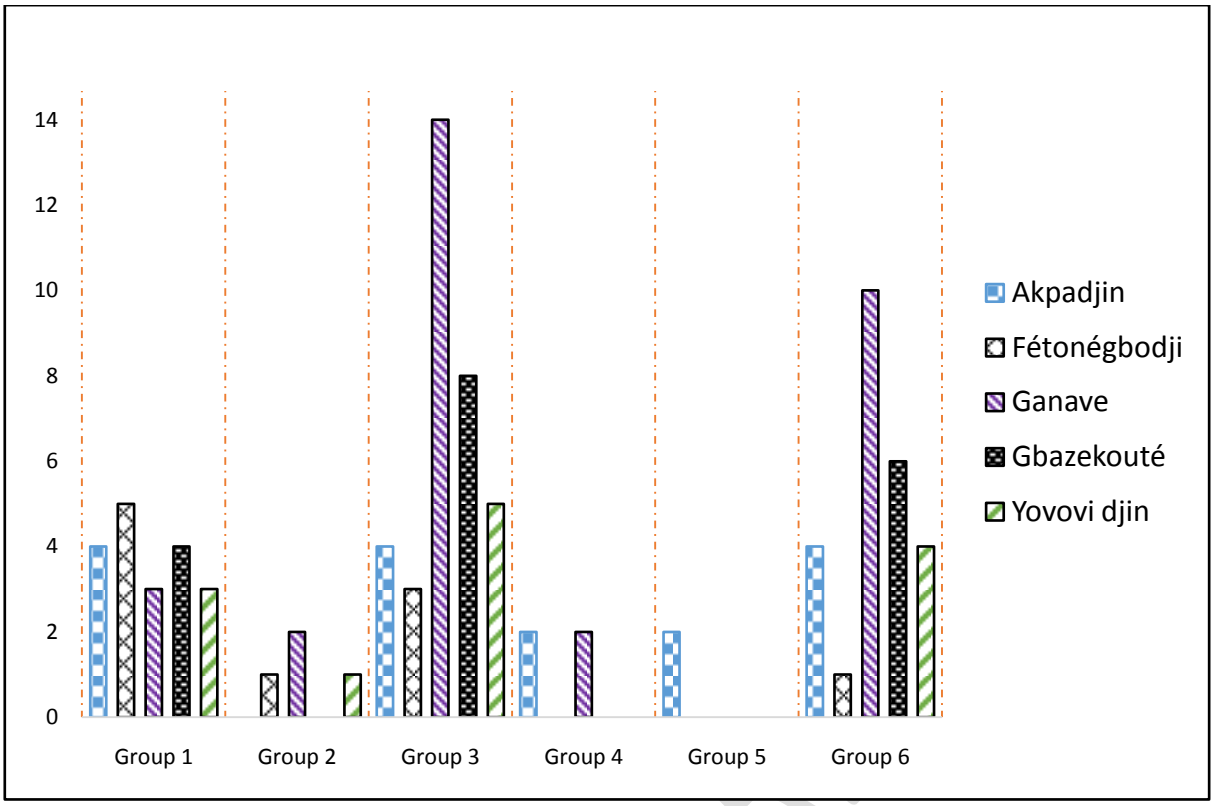

Figure 4 : Distribution of cultivars withingenetic groups

Analyses of the phylogenetic tree (Figure 3) and of the distribution of cultivars within genetic groups (Figure 4) showed that the genetic groups created contain individuals of different cultivars. Even individuals of the cultivar Gbazekouté, an improved variety, did not form homogeneous genetic groups.

These analyses show that some producers have confused the names of varieties by giving a variety name that does not conform even in the exchange of cuttings. This was demonstrated by Bajracharya et *al*. (2006) who reported that the concept of variety does not always correspond to the biological reality in family agrosystems. Also, producers of different ethnicities, or sometimes of the same ethnicity, use different names for the same varieties as reported by (Nuijten&Almekinders, 2008).

The analyses also showed that the majority of individuals from the Ganave, Gbazekouté and Yovovidjin cultivars were found in groups 3 and 6. This could be due to a low level of divergence (similarity) between individuals of these three cultivars despite the great diversity noted in the populations studied.

CONCLUSION AND PERSPECTIVES

The general objective of this study was to provide information on the genetic and molecular diversity of cassava cultivars collected in the Kara region using SSR microsatellite markers. It was found that the polymorphic information content (PIC) of the microsatellite markers used were very informative (PIC > 0.5) for the characterization of cassava cultivars. A lower genetic heterogeneity between populations (1 %) than within individuals of cultivars (12 %) was observed. The genetic structuring model based on the *Neighbour-Joining* algorithm method divided the analyzed individuals into 6 groups and showed no apparent relationship with the farmer nomenclature. In view of these results, the use of SSR is essential for the molecular characterization of cassava accessions and would be suitable for further studies on the genetic diversity of cassava in other economic regions of Togo. The results of this study also provide necessary background information for programs aiming at a better valorization of cassava.

REFERENCES

Adjebeng-Danquah, J., Gracen, V. E., Offei, S. K., Asante, I. K., & Manu-Aduening, J. (2016). Agronomic performance and genotypic diversity for morphological traits among cassava genotypes in the Guinea Savannah Ecology of Ghana. *Journal of crop Science and Biotechnology*, *19*(1), 99- 108.

Adjebeng-Danquah, J., Manu-Aduening, J., Asante, I. K., Agyare, R. Y., Gracen, V., & Offei, S. K. (2020). Genetic diversity and population structure analysis of Ghanaian and exotic cassava accessions using simple sequence repeat (SSR) markers. *Heliyon*, *6*(1), e03154. https://doi.org/10.1016/j.heliyon.2019.e03154

Agarwal, M., Shrivastava, N., & Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. *Plant cell reports*, *27*, 617- 631.

Anjum, M. A., Rauf, A., Bashir, M. A., & Ahmad, R. (2018). The evaluation of biodiversity in some indigenous Indian jujube (Zizyphus mauritiana) germplasm through physico-chemical analysis. *Acta Scientiarum Polonorum Hortorum Cultus*, *17*(4), 39- 52.

Bajracharya, J., Steele, K. A., Jarvis, D. I., Sthapit, B. R., & Witcombe, J. R. (2006). Rice landrace diversity in Nepal: Variability of agro-morphological traits and SSR markers in landraces from a high-altitude site. *Field crops research*, *95*(2- 3), 327- 335.

Carrasco, N. F. (2012). *Diversidade genética de variedades tradicionais de mandioca (Manihot esculenta Crantz) cultivada em comunidades da Baixada Cuiabana em Mato Grosso por meio de microssatélites* [PhD Thesis]. Universidade de São Paulo.

Ceballos, H., Kawuki, R. S., Gracen, V. E., Yencho, G. C., & Hershey, C. H. (2015). Conventional breeding, marker-assisted selection, genomic selection and inbreeding in clonally propagated crops: A case study for cassava. *Theoretical and Applied Genetics*, *128*(9), 1647‑1667.

Colombo, C., Second, G., & Charrier, A. (2000). Diversity within American cassava germ plasm based on RAPD markers. *Genetics and Molecular Biology*, *23*, 189‑199.

FAOSTAT. (2020). https://www.fao.org/faostat/en/#data

Kalia, R. K., Rai, M. K., Kalia, S., Singh, R., & Dhawan, A. K. (2011). Microsatellite markers : An overview of the recent progress in plants. *Euphytica*, *177*(3), 309‑334.

Kombate, K., Dansi, A., Dossou-Aminon, I., Adjatin, A., Kpemoua, K., Dansi, M., Koffi, A., & Sanni, A. (2017a). Diversity of Cassava (*Manihot esculenta* Crantz) Cultivars in the Traditional Agriculture of Togo. *International Journal of current research in Biosciences and Plant Biology*, *4*, 98‑113. https://doi.org/10.20546/ijcrbp.2017.406.012

Kombate, K., Dossou-Aminon, I., Dansi, A., Adjatin, A., Dassou, A., Kpemoua, K., Dansi, M., Koffi, A., & Sanni, A. (2017b). Cassava (*Manihot esculenta* Crantz) Production Constraints, Farmers' Preference Criteria and Diversity Management in Togo. *International Journal of Current Microbiology and Applied Sciences*, *6*, 3328‑3240. https://doi.org/10.20546/ijcmas.2017.606.391

Kouakou, D. A., Koffi, K. K., Bonny, S. B., Koffi, G. K., & Bi, A. I. Z. (2022). Assessment of Genetic Diversity in Côte d'Ivoire Cassava (*Manihot esculenta* Crantz) Using SSR Markers. *Philippine Journal of Science*, *151*(3), 1067‑1075.

Liu, K., & Muse, S. V. (2005). Power Marker : An integrated analysis environment for genetic marker analysis. *Bioinformatics*, *21*(9), 2128‑2129.

Mba, R. E. C., Stephenson, P., Edwards, K., Melzer, S., Nkumbira, J., Gullberg, U., Apel, K., Gale, M., Tohme, J., & Fregene, M. (2001). Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: Towards an SSR-based

molecular genetic map of cassava: *Theoretical and Applied Genetics*, *102*(1), Article 1. https://doi.org/10.1007/s001220051614

Mühlen, G. S., Martins, P. S., & Ando, A. (2000). Variabilidade genética de etnovariedades de mandioca, avaliada por marcadores de DNA. *Scientia Agricola*, *57*, 319‐328.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the national academy of sciences*, *70*(12), 3321‐3323.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, *89*(3), 583‐590.

Nosil, P., Egan, S. P., & Funk, D. J. (2008). Heterogeneous Genomic Differentiation Between Walking-Stick Ecotypes: "Isolation by Adaptation" and Multiple Roles for Divergent Selection. *Evolution*, *62*(2), 316‐336. https://doi.org/10.1111/j.1558-5646.2007.00299.x

Nuijten, E., & Almekinders, C. J. (2008). Mechanisms explaining variety naming by farmers and name consistency of rice varieties in the Gambia. *Economic Botany*, *62*(2), 148‐160.

Perrier, X., Flori, A., & Bonnot, F. (2003). Data analysis methods. *Genetic diversity of cultivated tropical plants*, *43*, 76.

Porth, I., & El-Kassaby, Y. A. (2014). Assessment of the genetic diversity in forest tree populations using molecular markers. *Diversity*, *6*(2), 283‐295.

Reynolds, S. M. (2017). *SNP-based genetic linkage mapping and whole-genome diversity assessment in cassava (Manihot esculenta Crantz) accessions from Africa and Latin America* [PhD Thesis]. University of Pretoria.

Santos Mühlen, G., Alves-Pereira, A., Clement, C. R., & Losada Valle, T. (2013). Genetic diversity and differentiation of Brazilian bitter and sweet manioc varieties (*Manihot esculenta* Crantz, Euphorbiaceae) based on SSR molecular markers. *Tipití: Journal of the Society for the Anthropology of Lowland South America*, *11*(2), 66‐73.

Singh, R. B., Singh, B., & Singh, R. (2018). Evaluation of Genetic Diversity in *Saccharum* Species Clones and Commercial Varieties Employing Molecular (SSR) and Physiological Markers. *Indian Journal of Plant Genetic Resources*, *31*(1), 17. https://doi.org/10.5958/0976-1926.2018.00003.7

Singh, R. B., Singh, B., & Singh, R. K. (2019). Development of potential dbEST-derived microsatellite markers for genetic evaluation of sugarcane and related cereal grasses. *Industrial Crops and Products*, *128*, 38‐47.

Smouse, R. P. P., & Peakall, R. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, *28*(19), 2537‐2539.

Tize, I., Fotso, A. K., Nukenine, E. N., Masso, C., Ngome, F. A., Suh, C., Lendzemo, V. W., Nchoutnji, I., Manga, G., & Parkes, E. (2021). New cassava germplasm for food and nutritional security in Central Africa. *Scientific Reports*, *11*(1), 1‐12.

Turyagyenda, L. F., Kizito, E. B., Ferguson, M. E., Baguma, Y., Harvey, J. W., Gibson, P., Wanjala, B. W., & Osiru, D. S. O. (2012). Genetic diversity among farmer-preferred cassava landraces in Uganda. *African Crop Science Journal*, *20*(1).

Varshney, R. K., Graner, A., & Sorrells, M. E. (2005). Genic microsatellite markers in plants : Features and applications. *TRENDS in Biotechnology*, *23*(1), 48‐55.

Wright, S. (1978). Variability within and among natural populations. *Evolution and the Genetics of Populations*.